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Letter to the Editor

Clinical efficacy of nitric oxide nasal spray (NONS) for the treatment of mild COVID-19 infection**Summary**

Baek et al.¹ investigated the duration of COVID-19 virus shedding in infected patients and demonstrated that even in patients demonstrating prolonged viral clearance, the virus was no longer viable after 15 days post onset of symptoms. Our study aimed to measure whether nitric oxide nasal spray (NONS) could accelerate the reduction in SARS-CoV-2 RNA load versus control with a saline spray. Our study recruited 80 participants who were divided into a NONS treatment or a placebo arm to test the efficacy of NONS as a treatment for mild COVID-19 infection.

Introduction

The coronavirus (COVID-19) pandemic has had a profound impact on the world, resulting in a worldwide death toll of over 2.6 million and global cases in excess of 119 million as at March 2021.² These figures demonstrate the necessity of rapidly developing new and effective ways in which to control and treat the virus in support of the emergency use of already-available COVID-19 vaccines.³

There are currently no evidence-based treatments for mild COVID-19 infection. This double-blind phase IIb clinical trial used a placebo control to evaluate the efficacy of nitric oxide in the treatment of mild, symptomatic COVID-19 infection in the form of a self-administered nasal spray. Nitric oxide (NO) is a free radical gas molecule involved in innate immunity, as well as wound healing, vasodilation, neurotransmission, and angiogenesis.⁴ Although produced physiologically, NO has been shown to exhibit a number of antimicrobial actions at therapeutic dosage regimens both *in vitro* and *in vivo*.^{5–7}

Materials and methods

This trial was carried out at Ashford and St. Peter's Hospitals NHS Foundation Trust (ASPHFT). 80 adults (18–70 years) who were isolated with mild COVID-19 infection confirmed by laboratory SARS-CoV-2 RT-PCR nasal and throat swab within the 48 h of randomisation were eligible for recruitment. Participants were randomised 1:1 to receive NONS ($n=40$) placebo ($n=40$). The nasal sprays were self-administered 5–6 times daily (two sprays per nostril/dose, 120–140 μ L of solution/spray) for 9 days.

Treatment with NONS or placebo commenced on day 1. Participants took self-sampled nasal and throat swabs on days 1 (at baseline, before initiating treatments), 2, 4, and 6 in the mornings,

prior to treatment. Quantitative RT-PCR was carried out at Berkshire Surrey Pathology Services Virology laboratory to determine SARS-CoV-2 RNA levels. SARS-CoV-2 sequencing for variants was performed at Public Health England Colindale. Daily self-reporting questionnaires on symptoms, compliance, and treatment tolerance were completed by patients and follow-up continues for a total of 18 days.

Results

Patients in both trial groups started on NONS or placebo at least 4 days after the onset of symptoms and were well balanced in terms of risk factors (Table 1). 34 (85%) of the NONS group and the placebo group were determined to be lineage B.1.1.7 (VOC20212/01) and the remainder were not determined to be a variant of concern. There were no serious adverse events in patients within either trial group. NONS versus placebo started on at least day 4 of symptom onset was independently associated with an accelerated decrease in \log_{10} SARS-CoV-2 RNA concentration of -1.21 (95% CI, -2.07 to -0.35 ; $P=0.01$) and -1.21 (95% CI, -2.19 to -0.24 ; $P=0.02$) on days 2 and 4 respectively (Fig. 1). Mean SARS-CoV-2 RNA concentration was lower on NONS by a factor of 16.2 at days 2 and 4. A rapid reduction (95%) in the SARS-CoV-2 viral load was observed within 24 hours, with a 99% reduction observed within 72 hours with NONS treatments.

The mean SARS-CoV-2 RNA concentration at day 6 was lowered to -3.32 on NONS, with a treatment difference of -0.98 (95% CI,

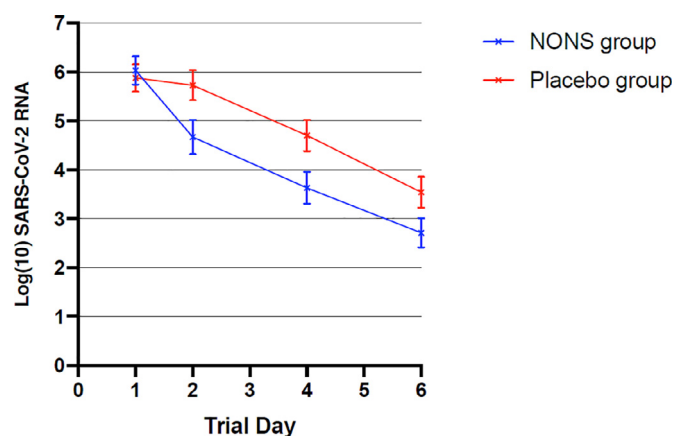


Fig. 1. Mean \log_{10} SARS-CoV-2 RNA at days 1 to 6. Shown is the difference in the change from baseline in SARS-CoV-2 RNA between the active (NONS) group and the placebo (saline) group from day 1 to day 6. The I bars represent standard error.

Table 1
Characteristics of the patients at baseline.

	NONS group (n = 40)	Placebo group (n = 40)	p-value
Age (mean, sd)	44 (12.1)	43.9 (12.6)	0.966
Sex			
Male	16 (40.0%)	13 (32.5%)	0.488
Female	24 (60.0%)	27 (67.5%)	
Ethnicity			0.692
White	34 (85.0%)	37 (92.5%)	
Black African + Caribbean	1 (2.5%)	0 (0%)	
South Asian	2 (5.0%)	3 (7.5%)	
Mixed	2 (5.0%)	0 (0%)	
Other	1 (2.5%)	0 (0%)	
BMI			0.034*
BMI ≥ 30	12 (30.0%)	7 (17.5%)	
BMI < 30	19 (47.5%)	30 (75.0%)	
No data	9 (22.5%)	3 (7.5%)	
Comorbidities			
Any comorbidity	6 (15.0%)	4 (10.0%)	0.502
Chronic lung disease	2 (5.0%)	0 (0%)	0.155
Chronic liver disease	0 (0%)	0 (0%)	
Chronic heart disease	0 (0%)	0 (0%)	
Diabetes	3 (7.5%)	2 (5.0%)	0.646
Hypertension	2 (5.0%)	3 (7.5%)	0.646
Presenting symptoms			
Dry cough	22 (55.0%)	27 (67.5%)	0.496
Fever	7 (17.5%)	16 (40.0%)	0.081
Loss of sense of smell	9 (22.5%)	5 (12.5%)	0.323
None of the above	6 (15.0%)	8 (17.5%)	0.743
No data	5 (12.5%)	3 (7.5%)	
SARS-CoV-2 variant			
B.1.1.7	34 (85.0%)	34 (85.0%)	
Not known variant	6 (15.0%)	6 (15.0%)	

−2.04 to 0.08; $P=0.069$). The mean treatment difference using an area under curve estimate from baseline through day 6 was −5.22 with a 95% CI, −9.14 to −1.31; $P=0.001$), where the mean change was −10.17 for the NONS group and −4.95 for the placebo group.

40 subjects (15 NONS and 25 placebo subjects) completed and returned the trial assessment questionnaire. A total of 46.7% (7 of 15) of NONS respondents reported feeling better versus 8% (2 of 25) of placebo respondents on treatment. NONS subjects typically reported being better by day 2–4 on treatment, whereas the placebo subjects typically did not report feeling better until after day 5.

Discussion

Treatment with NONS in this trial was found to be effective and safe in reducing the viral load in patients with mild, symptomatic COVID-19 infection. Patients with recent disease onset were enrolled in the trial to evaluate the effect of early intervention with NONS on SARS-CoV-2 RNA load. Patients in the NONS treatment arm demonstrated viral loads, as determined from PCR testing of nose and throat swab sampling, that were lower at days 2 and 4 by a factor of 16.2 than those on placebo, and symptom resolution was also found to be faster on NONS treatment than on placebo in this study.

Lower SARS-CoV-2 RNA loads in patients with NONS may be beneficial in the prevention of SARS-CoV-2 transmission. It has been described that higher viral loads in patients with SARS-CoV-2 earlier than SARS-CoV may have contributed to greater difficulties in reducing the onward transmission.⁸ Furthermore, it has been observed that the risk of symptomatic COVID-19 was associated with the SARS-CoV-2 RNA levels of contacts and incubation time was shortened in a dose-dependent manner.⁹

Accelerated SARS-CoV-2 clearance with NONS may reduce symptom duration, decrease infectivity period, reduce hospital

admissions, and lower disease severity. Consequently, this study could be used as supporting evidence for emergency use of NONS for patients with mild COVID-19 infection.

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